AD_____

AWARD NUMBER: W81XWH-14-1-0265

TITLE: The Role of TREM2 in Traumatic Brain Injury Induced Tauopathy

PRINCIPAL INVESTIGATOR: Bruce T. Lamb

RECIPIENT: Cleveland Clinic Foundation
Cleveland, OH 44195

REPORT DATE: September 2015

TYPE OF REPORT: Annual report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
September 2015	Annual	15 Aug 2014 - 14 Aug 2015

4. TITLE AND SUBTITLE
The Role of TREM2 in Traumatic Brain Injury Induced Tauopathy

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0265

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Bruce T. Lamb

Email: lambb@ccf.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
.
Cleveland Clinic Foundation
Cleveland, OH 44195

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Traumatic brain injury (TBI) promotes several Alzheimer's disease (AD)-like pathological features including accumulation of microtubule-associated protein tau (MAPT) within neurons; however, genetic risk factors that link TBI and MAPT pathology remain unclear, The recent identification of mutations in Triggering Receptor Expressed on Myeloid cells 2 (TREM2) that are associated with dementia and AD enables studies to examine the relationship between TBI, inflammation, TREM2 and development of AD-like pathologies expressed in a mouse Mapt knockout background19. We have previously shown that TBI causes enhanced MAPT phosphorylation and aggregation with heightened macrophage activation in hTau mice at 3 DPI suggesting that there is a correlation between MAPT pathology and alterations in macrophage activation following TBI. We hypothesize that up-regulation of TREM2 within monocytes and microglia observed following TBI plays a protective role in the development of MAPT pathologies. These studies will develop novel resources and models to identify potential genetic factors regulating the TBI-AD connection as well as pathways downstream of TREM2 that may impact TBI induced MAPT pathology and neurodegeneration. Indeed, this work is particularly relevant for high risk TBI populations such as soldiers, as potential therapies focused on TREM2/monocytes/microglia can be targeted in future studies.

15. SUBJECT TERMS-

Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	15	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Traumatic brain injury (TBI) promotes several Alzheimer's disease (AD)-like pathological features including accumulation of microtubule-associated protein tau (MAPT) within neurons; however, genetic risk factors that link TBI and MAPT pathology remain unclear, The recent identification of mutations in *Triggering Receptor Expressed on Myeloid cells 2 (TREM2)* that are associated with dementia and AD enables studies to examine the relationship between TBI, inflammation, TREM2 and development of AD-like pathologies expressed in a mouse *Mapt* knockout background[19]. We have previously shown that TBI causes enhanced MAPT phosphorylation and aggregation with heightened macrophage activation in hTau mice at 3 DPI suggesting that there is a correlation between MAPT pathology and alterations in macrophage activation following TBI. ***We hypothesize that up-regulation of TREM2 within monocytes and microglia observed following TBI plays a protective role in the development of MAPT pathologies.*** These studies will develop novel resources and models to identify potential genetic factors regulating the TBI-AD connection as well as pathways downstream of TREM2 that may impact TBI induced MAPT pathology and neurodegeneration. Indeed, this work is particularly relevant for high risk TBI populations such as soldiers, as potential therapies focused on TREM2/monocytes/microglia can be targeted in future studies.

2. KEYWORDS: Provide a brief list of keywords (limit to 20 words).

TREM2, Alzheimer's Disease, Traumatic Brain Injury, MAPT pathology, Neuroinflammation, neurodegeneration, Fluid percussion injury, Microglia

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Approvals and Training of Staff
Task 1. Complete approval documents for the studies (timeframe, months 1-4) 100% Complete
Task 2. Provide required training for staff (timeframe, months 1-4) 100% Complete
Specific Aim 1
Task 1. Generate animals required for studies (timeframe, months 2-6) 100% Complete
Task 2. Perform FPI (timeframe, months 6-10) 100% Complete
Task 3. Perform behavioral analysis (timeframe, months 10-16) 80% Complete
Task 4. Analysis of brain tissue (timeframe, months 10-16) 80% Complete
Task 5. Published manuscript on Specific Aim 1 (timeframe, months 16-20) 50% Complete
Specific Aim 2
Task 1. Generate animals required for studies (timeframe, months 6-12) 80% Complete
Task 2. Perform FPI (timeframe, months 12-16) 20% Complete
Task 3. Perform behavioral analysis (timeframe, months 16-22) 0% Complete
Task 4. Analysis of brain tissue (timeframe, months 16-22) 0% Complete
Task 5. Flow cytometry, gene expression analysis, and multiphoton microscopy (timeframe, months 18-24) 0% Complete

Task 6. Published manuscript on Specific Aim 2 (timeframe, months 24-28) 0% Complete

Specific Aim 3

Task 1. Generate animals required for studies (timeframe 14-22 months) 50% Complete

Task 2. Perform FPI (timeframe, months 22-26) 0% Complete

Task 3. Preform behavioral analysis (timeframe, months 26-32) 0% Complete

Task 4. Analysis of brain tissue (timeframe, months 26-32) 0% Complete

Task 5. Published manuscript on Specific Aim 3 (timeframe, months 32-36) 0% Complete

What was accomplished under these goals?

1) This work has been presented at multiple local and national conferences. The majority of the work focuses at looking at inflammation acutely using Immunohistochemistry and behavior chronically.
2) Our specific objective was to look at the role of TREM2 in traumatic brain injury-Induced (acute) neuroinflammation and (chronic) neurodegeneration
3) Our major findings can be found below.
4) Ms. Saber also received a travel grant from society of Neurotrauma to present this work at their conference in Santa Fe (June 2015)

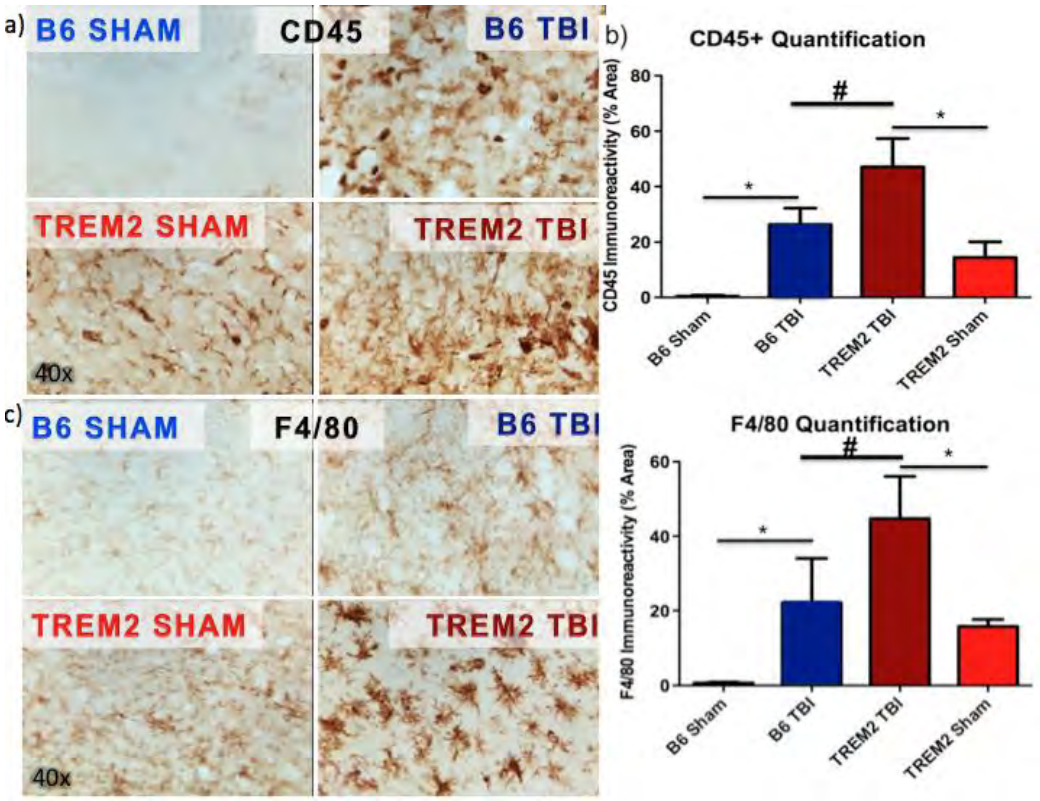

Figure 1. Increase in inflammatory cells found lateral to the lesion at 3DPI. Heterozygous and homozygous Trem2 knockouts were sacrificed at 3 Days Post Injury (DPI) to look at acute effects of injury. Currently, staining has been done for Trem2-/- and B6 mice for CD45 and F4/80. Staining and quantification is shown above. a) Representive images for CD45 staining, an immune cell marker. b) Quantification of CD45 staining. c) Representive images for F4/80 staining, a macrophage marker. d) Quantification of F4/80 staining. We will finish staining for Trem2+/- and look at other proteins. Brain samples will also be collected at 120DPI for immunohistochemistry or biochemical analysis.

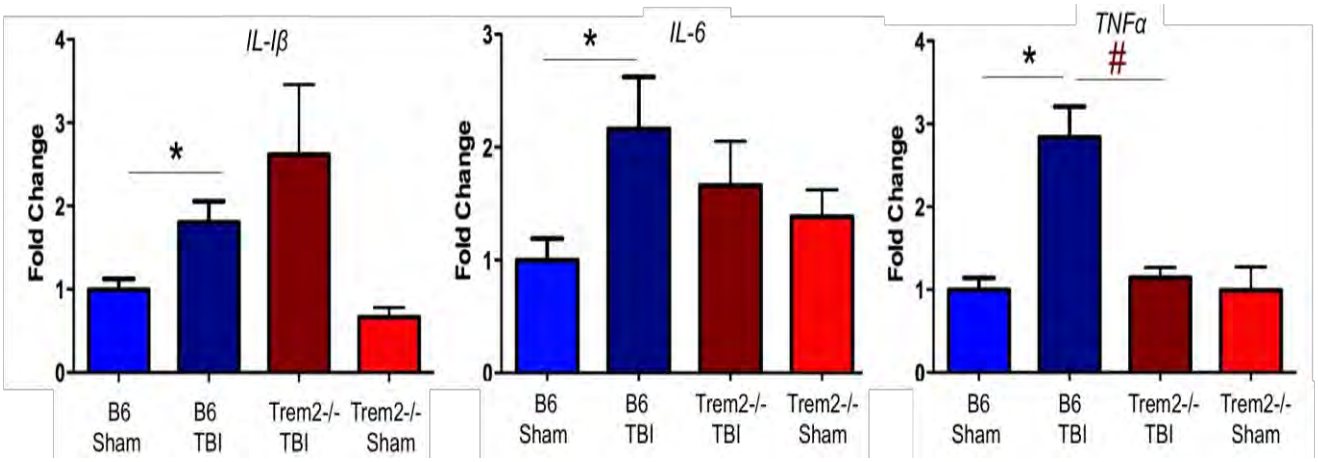

Figure 2. Decrease in select inflammatory cytokines in TREM2-/- mice after 3DPI. Heterozygous and homozygous Trem2 knockouts were sacrificed at 3 Days Post Injury (DPI) to look at acute effects of injury. Using qPCR to measure mRNA production of inflammatory cytokines, we saw a reduction in TNFalpha in Trem2 deficient mice after TBI 3DPI. We will follow this finding up by measuring protein expression using western blots and by measuring the levels of other cytokines.

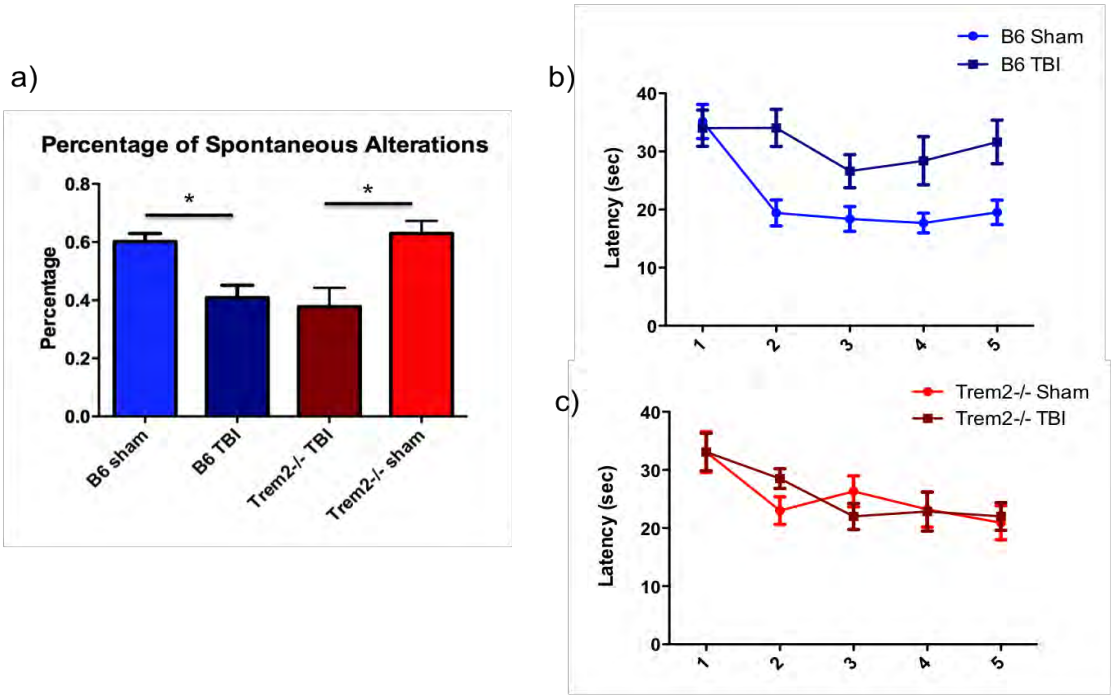

Figure 3. Changes in chronic behavior after TBI. a) We saw in both b6 and TREM2-/- mice that there was a change in the percentage of spontaneous alternations after a chronic (90DPI) TBI. b) This was not genotype specific and seen in both genotypes. B6 mice showed a significant deficiency in locating the hidden platform during watermaze testing after TBI compared to sham treated mice ($p<.05$). c) However, there was no significant change in latency to reach the platform between the Trem2-/- TBI group versus Trem2-/- sham group. (*$p<.05$)

What opportunities for training and professional development has the project provided?

This project has provided the trainees multiply opportunities for professional development. This project has allowed Dr. Kokiko-Cochran to practice her mentoring skills with Ms. Saber. She has taught Ms. Saber how to perform all surgeries and experiments used in this project. Ms. Saber has also gone to workshops that will allow her to understand the more detailed concepts in this work. For example, Ms. Saber went to an Alzheimer's disease (AD) workshop hosted by the BrightFocus foundation to strengthen her critical thinking in neurodegenerative diseases.

No outreach activities were undertaken but this work has been presented on at multiple different local and national conferences.

We plan on following our timeline as closely as possible. We will try to complete the majority of surgeries required on this grant within the next quarter. We will continue to run behavior and analyze acute and available chronic brain tissue.

4. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

What was the impact on the development of the principal discipline(s) of the project?

TREM2 has been shown to be involved in multiple different inflammatory diseases; however, the exact role of TREM2 is not yet known. Multiple studies looking at the role of TREM2 in disease states, both in cells and in mice, have shown very contradictory results. These results have suggested that the role of TREM2 may be different in different pathological contexts. Our goal is to see what the role of TREM2 is in the context of TBI and whether that can be linked to neurodegeneration and AD pathology.

What was the impact on other disciplines?

These findings should impact not only the TBI field, but the immunology and neurodegeneration fields as well. The role of TREM2 has been canonically thought to be anti- inflammatory in the immunology field. However, in the neurodegeneration field the findings have been more controversial. Discovering the role of TREM2 in TBI will add more information in finding what exactly TREM2 does and how it effects neurodegeneration.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

TBI is currently a hot topic in the media. Many studies have shown the potential link of TBI to neurodegeneration

5. **CHANGES/PROBLEMS:** The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

The Lamb lab will be transferring from the Cleveland clinic to Indiana University School of Medicine in Indianapolis in January. We expect a few months delay in being able to complete this project as the new lab is set up, although we will aim to reduce this delay as much as possible through advanced planning. In anticipation of the move we have started surgeries for animals in both Specific Aim 2 and 3. Some preliminary data has been generated for both acute and chronic time points for specific aim 3. We plan to complete as many surgeries as possible until January and using the time where the lab is restarting to analyze data.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

6. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

- **Publications, conference papers, and presentations**

Report only the major publication(s) resulting from the work under this award.

Journal publications.

Nothing to Report

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Saber M, Kokiko-Cochran ON., Teknipp R., Miller C.,Lamb BT. "The Role of TREM2 in Traumatic Brain Injury-
Induced Neuroinflammation and Neurodegeneration" CCLCM, Case graduate symposium, 05/2015 (Poster)
Saber M, Kokiko-Cochran ON., Teknipp R.,Hales J.,Lamb BT. " The Role of TREM2 in Traumatic Brain
Injury-Induced Neuroinflammation and Neurodegeneration" CCLCM, LRI Day, 05/2015 (Poster)
Saber M, Kokiko-Cochran ON., Teknipp R.,Hales J.,Lamb BT. " The Role of TREM2 in Traumatic Brain
Injury-Induced Neuroinflammation and Neurodegeneration" Society of Neurotrauma (Sante Fe), 06/2015 (Poster)

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Bruce T. Lamb
Project Role: Principal investigator
Researcher Identifier:
Nearest person month worked: 1.2 months
Contribution: Dr. Lamb oversaw the progress of the project and directed Dr. Kokiko- Cochran and Ms. Saber to complete the project accurately and on time.

Name: Olga Nicole Kokiko- Cochran
Project Role: Post-doctoral fellow
Researcher Identifier:
Nearest person month worked: 7.5 months
Contribution: Dr. Kokiko- Cochran has primarily worked on Specific aim 2 and 3. She has done all surgeries and has generated some preliminary data for both specific aims. Dr. Kokiko- Cochran has trained Ms. Saber on completed all surgeries and experiments.

Name: Maha Saber
Project Role: Graduate student
Researcher Identifier: 795314
Nearest person month worked: 5 months
Contribution: Ms. Saber has primarily worked on Specific aim 1 and has assisted Dr. Kokiko with surgeries for specific aim 2 and 3. Ms. Saber has completed all surgeries and behavior required for specific v Aim 1 and is currently finishing brain tissue analysis for

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

COMPLETED

W81XWH12-1-0629 (Lamb, BT) 10/1/12-9/30/14 0.6 calendar
United States Department of Defense $500,000.00

Novel Genetic Models to Study the Role of Inflammation in Injury-Induced AD

The overall goal of this application is to examine the effects of traumatic brain injury on Alzheimer's disease pathologies at both early (3 DPI) and late (120 DPI) time points. First, the effects of TBI on extracellular amyloid pathology will be examined in a genomic-based mouse model of AD. Second the effects of TBI on intracellular tau aggregation will be examined in a genomic-based mouse model of AD. Finally, the effects of TBI on the infiltration of monocytes and microglia will be examined at both the early (3DPI) and late (120 DPI) time points.

NEWLY APPROVED

R01 AG051495 (Lamb) 9/30/15 -8/31/20 2.4 calendar
NIH/NIA $2,500,000 total DC

Central and Peripheral Role of TREM2 in Alzheimer's Disease

Multiple recent studies have demonstrated that the *TREM2* gene, and innate immunity more generally, plays a critical role in the pathogenesis of neurodegenerative diseases, including AD. However, it remains unclear in which cell populations (microglia, monocytes, etc.) TREM2 acts, the exact role of TREM2 in regulating neurodegenerative disease pathologies. The current interdisciplinary studies seek to examine whether brain resident microglia and blood-derived $TREM2^{+}$ monocytes play distinctive roles in regulating AD pathologies and whether the TREM2+ cell population could provide novel biomarkers/diagnostics and be targeted therapeutically.

What other organizations were involved as partners?

Nothing to report